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CONTENTS

VOLUME EIGHTEEN NUMBER 4

JULY-AUGUST

1952

Page

MEANS OF CONTROL OF CHEMICAL PROCEDURES 183
Esther F. Freier, B.S., M.T. (ASCP)

EXPERIENCE WITH Rh SENSITIVITY TITERS 187
Lydia Brownhill, M.T. (ASCP)

A SPECIFIC HEMAGGLUTINATION TEST IN THE DIAGNOSIS OF TUBERCULOSIS 190
Frances G. Murry, M.T. (ASCP)

CHANGES FOUND IN BLOOD AND HEMATOPOIETIC FUNCTION IN CHRONIC LEAD EXPOSED PAINTERS.. 195
Wilhelmina Schroeder, M.T. (ASCP)

COCCIDIOIDOMYCOSIS IN SOUTH TEXAS; CASES OBSERVED IN SANTA ROSA HOSPITAL..... 199
Frederick W. Bieberdorf, Ph.D.

DETERMINATION OF ANTISTREPTOLYSIN TITERS: TECHNIC AND SIGNIFICANCE 205
Ruth Robinette, B.S., M.T. (ASCP)

EXPERIENCE WITH A BONE BANK OPERATED AT A VETERANS ADMINISTRATION HOSPITAL 214
Norma J. Cambron, M.T. (ASCP)

A METHOD FOR RECORDING LABORATORY PROCEDURES 217
Alice T. Strozik, M.T. (ASCP)

THE GAVEL 218

AMONG THE NEW BOOKS..... 219

ABSTRACTS 221

ANNOUNCEMENTS 225

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MEANS OF CONTROL OF CHEMICAL PROCEDURES*

ESTHER F. FREIER, B.S., M.T. (ASCP)
Instructor Chemistry Laboratory, University of Minnesota Hospitals

Those of you who work for one doctor in an office probably do not have any trouble deciding whether your results are right or not. If they don't match his diagnosis, you hear about it. In a hospital, however, there are many doctors, sometimes with differing diagnoses for the same patient. It wouldn't do, however, to decide whether the results are right or not by how closely they match the clinical diagnosis. We, as medical technologists, are responsible to the doctor and to the patient for the reliability of our results, so we should be the ones to decide whether they are accurate or not. How? By policing and controlling our procedures. Particularly in a chemistry laboratory, we have in our power the means of proving the validity and reliability of our results without waiting to ask the doctor if they match the clinical picture. Let us examine the means of control of clinical chemical procedures from the standpoint of the type of information that each can give us.

Duplicates:

This is a check on technique—replicates you'll remember, check the **precision** of a method, not its **accuracy.** It is possible to have duplicates agree and still have an inaccurate result on a colorimeter determination if the standard concentration is off. So duplicates alone won't tell us whether the results are right or not. Assuming that we can ignore the question of precision of replicate pipetting manipulations by a trained technologist, the precision of duplicates can give us valuable information on the condition of equipment. For example:

* First Award. Minnesota S.M.T. paper-writing contest. Reprinted by permission from the Minnesota Medical Technologist, Jan. 1952.

1. Calibration of colorimeter tubes.
   If our tubes are not matched exactly, our duplicates can agree no more closely. Dirty or scratched colorimeter tubes can cause trouble here, too.
2. A leaky burette or a dirty one will not give good duplicate titrations.
3. In the author's laboratory, in addition to the usual checking of the Van Slyke Volumetric apparatus for leaks, each determination is checked for leaks by re-evacuating, reshaking and rereading the same gas volume. The agreement is within .01 ml if no leak is present.

In general, we can say that the practice of including a duplicate in every batch will tell us how precise the other results in that batch are; but we must look to further checks and controls to find out whether or not these results are accurate.

Standards:

Running a standard along with every batch will enable us to compensate for variables such as time, temperature, age of reagents, etc., on the theory that these variables will affect the standard and unknown to the same degree. One standard point alone should be enough to calculate a K value which is the slope of the standard line, but having several standard points is an additional check on technique. In essence, we are calibrating our colorimeters every time we run a standard curve. Needless to say, the use of permanent graphs and tables (the advertising piece *de resistance* of the companies selling pre-calibrated colorimeters) without running a standard to check the reagents can lead to serious trouble. If a variable, such as the temperature of a boiling water bath varying with barometric pressure, is involved in the procedure, at least one standard must be included in every batch. Comparing standard reading from day to day will enable you to catch errors, such as reading with the wrong filter.

It is possible to run duplicates and standards and still be turning out inaccurate results. The concentration of the standard may be off. Most of us remember that glucose standard is unstable, particularly in warm weather. While the stock standard solutions are usually stable, we have to be alert to the fact that they may not be stable in the more dilute working solutions. If the standard has deteriorated so that it no longer is as concentrated as we think it is, the determinations will be falsely high. This can be checked by making a new dilution from the stable stock solution.

Recoveries:

A recovery will tell us how good the method itself really is. If we add a known amount of the thing we're measuring, can we

recover it as an added increment of the unknown? The fact that a given method doesn't give a 100% recovery, in itself, should not be a deterrent to the use of the method. For example, in the urine creatine method, some of the preformed creatinine is destroyed during the heating which converts creatine to creatinine, so the recoveries average about 85-90%, but we can establish normals and use the method clinically as long as the recovery is consistent.

Recovery of added urea in the Blood Urea Nitrogen methods which use the enzyme urease, serves to check the potency of the activity of the enzyme preparation. Since this enzyme activity can vary with time and temperature of incubation, a recovery should be included in every run of this procedure. On many other procedures, a recovery would give no more information than another standard concentration and should be run when the accuracy of the method is under scrutiny or when a new procedure is being evaluated, rather than as a routine practice.

A Reference Method:

Results can also be checked by running the determination with another established method which may not be practical for routine use because it is time-consuming. For example, the micro Kjeldahl is considered to be the most valid method for the determination of total serum proteins, but it requires too much time for routine clinical use. However, should the validity of the protein results obtained with one of the convenient colorimetric methods be questioned, a Kjeldahl could be run on one of the specimens to see if there was a discrepancy between the two methods.

The reference method can measure a parallel blood constituent and still serve as a check on a given procedure. The practice of running creatinines on specimens with high blood urea nitrogens serves as a check on the blood urea nitrogen procedure, and occasionally will catch a mixup in specimens.

Normal Controls:

Including a normal specimen along with our run is another means of checking whether our results are valid or not. However, since the physiological range may be greater than the degree of error we expect in a given procedure, we may find that we can have inaccurate results and still have a normal specimen coming out in the normal range. Then, unless we want to bleed ourselves continuously, we start asking the wards to send in normal controls and occasionally we find that the patient they select for a control may be more abnormal than the unknown.

Frozen Pooled Serum Controls:

A pooled serum can be used as a control, eliminating the drawbacks mentioned above for a normal control. The pooled serum should be divided into convenient aliquots for each day's use and stored in a deep freeze or freezing compartment of a refrigerator. This will eliminate the effects of repeated freezing and thawing of the same specimen. Each day the control aliquot is thawed, **mixed well,** and centrifuged before use. The comparison of the value of the control on a given day with the established statistical mean of that pooled serum will give us a measure of the accuracy of the results on that day.

The control can indicate when reagents or standards have deteriorated and are affecting the results. It will tell us when our standard has deteriorated because we will then obtain a higher value for the pooled serum control. The use of these frozen controls is based on the assumption that the value of the control doesn't change with storage in the frozen state. Therefore a change in value of the control must indicate trouble somewhere in the procedure. Charting the control values graphically from day to day in the manner described by Levey & Jennings[1] enables one to spot when a method goes out of control sooner than looking through a protocol book occasionally for the control data. A new pooled serum control should be started before the old one is exhausted, so the mean for the new control can be established before it must be used to check the method.

Errors That Are Not Controlled:

I would like to close this discussion with a reminder that results can be invalid even though we were to use all of these means of policing our chemical methods. Errors such as mixing up blood specimens, misreading the colorimeter or burette, putting the readings down opposite the wrong patient's name, or using a 2 cc pipette instead of a 1 cc pipette are still possible. Proper awareness of our responsibility to the patient should provide the care necessary to avoid this type of error.

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Technical Bulletin of the Registry of Medical Technologists (ASCP) **20,** 1059, November, 1950.

EXPERIENCE WITH Rh SENSITIVITY TITERS

LYDIA BROWNHILL, M.T. (ASCP)*
1079 East Main St., Meriden, Conn.

Since the discovery of the Rh factor in 1940 by Landsteiner and Wiener, many procedures have been brought forth in the field of Rh. Rh typing has long since become routine and with careful handling presents few problems. However, methods for Rh antibody titrations may become extremely confusing because, although the principles remain the same, there are several schools of thought as to the proper method of performing these tests.

It is generally agreed that a complete antibody titration method must test for univalent or incomplete antibodies, bivalent antibodies or agglutinins, and true blocking antibodies. In 1944 and 1945 Race[2] in England and Diamond and Denton[1] in this country discovered that not all Rh antibodies agglutinated in saline. Since that time numerous substitutes such as pooled plasma, human serum, acacia, and bovine albumin have been used in various combinations as the medium for washing cells and as the diluent in the titrations. From this array of possible media it is necessary for the technologist to choose a method for Rh sensitivity titers which is applicable to her needs.

In the past year we have been using a modification of the method set up by the Rh Typing Laboratory in Baltimore.[3] The original method requires an albumin, serum, saline, and indirect blocking system. We have felt that the serum titer, in which pooled type O Rh positive serum is used to suspend the cells is not practical for our use and with this omission we have found this method to be satisfactory and thoroughly reproducible.

All sera sent to us for antibody testing are set up in serial dilution using the albumin and saline systems with a single tube indirect blocking test. We do not use any kind of screening test because we feel that for our purposes it is just as easy to set up several dilutions in the first place as it is to do a screening test and then set up the dilutions. Also, we feel that there is an advantage in that the possibility of missing a prozone reaction is eliminated.

We have tested for univalent or incomplete Rh antibodies by washing and suspending pooled type O Rh positive cells in 30% bovine albumin using pooled Type O Rh positive serum as the diluent in the titrations. In this way no saline enters the test at any time. An amount of cell suspension is added equal to the amount of dilution present in the tube. That is, if there is 0.1cc. dilution in the test, 0.1cc. of cell suspension will be added. A positive control is set up using 0.1cc. of serum known to contain

*Read before ASMT, Swampscott, Mass., June, 1951.

Rh antibodies and 0.1cc. of the cell suspension. The negative control consists of 0.1cc. of the serum used as the diluent and 0.1cc. of cell suspension. This negative control acts as a check to be certain that no clumping is taking place between the diluent and the cell suspension. The test is incubated for one hour at 37°, centrifuged for two minutes at 1500 revolutions, and the amount of clumping noted.

The chief difficulty encountered using this method is in the viscosity of the albumin which is so great that the cells are not easily washed. This may be overcome by centrifuging at high speed for at least ten minutes at a time during the washing, and by withdrawing the supernatant with a pipette and suction so that few cells will be lost.

The saline system for bivalent or agglutinin antibodies is set up in the same fashion using normal saline as the medium for washing cells and as the diluent. These titers do not run as high as the albumin titers and unless the patient is known to have a high albumin titer, only one tube need be set up.

The indirect blocking system consists of 0.05cc. of patient's serum and 0.05cc. of saline cell suspension. The test is incubated for one hour and Rh antiD test tube typing serum is added. The test is reincubated for one hour, centrifuged gently and read for clumping. A control consisting of 0.05 cc. of cell suspension with Rh antiD typing serum, will serve to determine the maximum amount of clumping possible with that cell suspension. If the unknown shows an amount of clumping any less than the control, the blocking test is positive and the patient's serum must be set up in dilution and the test repeated. In patients known to have Rh antibodies the serum is serially diluted in saline and the blocking test run in dilution.

We have had a series of 35 cases in which we have found 28 cases with the highest albumin titer between 0 and 25 units and 7 in which the titer was positive 1:50 to 1000 and over. On these cases we have kept complete case histories and two of them are presented as examples.

The first is a case of a woman in her third pregnancy who, in her second pregnancy was delivered of an erythroblastotic infant. The infant was treated with an exchange transfusion and survived. In her third pregnancy the first Rh titer was taken in her sixth month and then monthly until the time of delivery. The baby was again an erythroblastotic infant who was exchange transfused with 1,000cc. of Rh negative blood from a female donor. The graph shows the titers on the mother and the baby during the exchanges.

This is the highest titer we have encountered, and despite it, the baby survived.

Case I

	7/5	7/22	8/5	9/5	10/14	10/16
Mother's Titer Albumin	1-1024	1-2048	1-16384	1-16384		1-32768
Saline	1-4	1-8	1-16	1-16		1-32

Baby's Titers	Albumin	Block A-b	Saline	Coomb's
Cord Blood	1-1024	1-4	1-8	
Pre-exchange	1-1024	1-2	1-4	1-8
280-300 cc.	1-128	1-1	—	1-8
480-500 cc.	1-64	1-1	—	1-8
Before 2nd exchange	1-32	—	—	1-4
280-300 cc.	1-8	—	—	1-2
480-500 cc.	1-2	—	—	1-1

Case II

	9/21	9/28	10/5	10/12	10/20			10/23
Mother's titer	1-4	1-32	1-64	1-64	1-64			1-128
Blocking anti-bodies				1-2	1-2			1-2

Baby's Titers	Albumin	Blocking Anti-Bodies	Coomb's
Cord Blood	1-64	1-2	1-8
Pre-exchange	1-128	1-1	1-8
280-300 cc.	1-8	—	1-8
480-500 cc.	1-2	—	1-1

The second case was of interest to us because, although the titers were not as high as we have seen in the other case, the reactions were very strong. Also the indirect blocking test appeared at a lower albumin titer than most other cases we have encountered. Although an exchange transfusion was performed on the day of delivery this baby expired.

We feel that this method fulfills all the requirements of a routine method for a small hospital laboratory. The instructions are relatively easy to follow, it takes no special training, and the reactions are so definite that the test is very easily read. The clear cut reaction is probably its most valuable feature because, since Rh antibody titers are not a daily procedure in a routine laboratory, it is essential that any positive reaction be very apparent.

In this series of 35 we have had no cases of low maternal Rh titers resulting in infants suffering from hemolytic disease. In all the cases with maternal titers higher than 1:32 the infants showed some evidence of hemolytic tendencies.

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A SPECIFIC HEMAGGLUTINATION TEST IN THE DIAGNOSIS OF TUBERCULOSIS*

By FRANCES G. MURRY
1325 South Grand Blvd., Saint Louis, Mo.

Introduction and Background

Some twelve or fifteen months ago, a stir of interest among lay people, as well as among the medical and allied professional personnel, was aroused by a report in Time magazine's medical section on a new laboratory test for tuberculosis—thought possibly to indicate the degree of activity of the disease. It was about that time that the investigations reported here were begun, and in the past year, it has been possible to gather and test blood sera from an interestingly well controlled group.

To briefly review the background for these investigations: In a letter to the editors of the American Review of Tuberculosis in 1948, Doctors Middlebrook and Dubos,[1] of the Rockefeller Foundation, published a note on their observations of a water-soluble component of extracts of the tubercle bacillus which could be firmly adsorbed onto sheep red blood cells, and these cells in turn could be agglutinated by the sera of rabbits previously immunized with injections of the tubercle bacillus.

Later that same year, these men[2] reported their procedures and methods in great detail. While the actual laboratory test was a relatively simple procedure, the preparation of their sensitizing antigen was involved and complicated enough to render that procedure practically prohibitive to the ordinary routine laboratory. However, in at least one specimen of Old Tuberculin, they had demonstrated the presence of a similar material.

A Doctor Scott and Miss Nancy Smith,[3] at Duke University, recognized the difficulties of preparing the sensitizing antigen, and working with Lederle Laboratories, found an Old Tuberculin suitable for use as a sensitizing agent. Prepared in four times standard strength, this Old Tuberculin seemed equally as specific as the previously described antigen. It was sufficiently cheap and eliminated the tedious details of preparation. These findings were reported early in 1950, and in the studies reported in this paper, the sensitizing agent was Old Tuberculin, four times standard strength, very generously supplied by Doctor H. D. Piersma, of Lederle Laboratories.

In changing from the original sensitizing agent to the Old Tuberculin, some slight modifications were made by Scott and Smith. Similar, and almost simultaneously, changes in the original technique were made by Doctor Sidney Rothbard.[4] These modifications were incorporated in the technique to be described here.

*Read before ASMT, Swampscott, Mass., June, 1951.

When the studies reported here were begun, all available information on previous works[5, 6] described the use of sheep red blood cells for sensitizing. Doctor Rothbard also described a modification for substituting the patient's own erythrocytes. In starting the studies, it was felt that some real advantages might be seen if the technique could be adapted to the use of human, type O cells. First, the anti-sheep factor present in a certain number of individuals would be by-passed; and second, the universal availability of human, type O, cells as compared to the necessity of securing sheep cells commercially, seemed a distinct advantage.

Methods

The details of the techniques were identical with sheep and human cells. Old Tuberculin was diluted 1 to 12 in buffered physiologic saline and the pH adjusted to 7.0 with 0.1N NaOH. To 10 cc. of the dilute Old Tuberculin, 0.5 cc. of well-washed packed erythrocytes were added in a round-bottom centrifuge tube, and incubated for two hours at 37° C. with frequent shaking. This particular tube was chosen to afford ample space for mixing, and as a convenient choice for washing the sensitized cells. During the incubation period, a sufficient quantity of the Old Tuberculin was adsorbed onto the cells to render them specifically sensitized to the serum factor demonstrated in these studies. After thorough washing, the cells were diluted to a 0.5 per cent suspension.

After inactivation, each serum was treated to remove any natural antibodies to sheep or human, type O, cells. Two tubes containing 2.0 cc. of a 1 to 2 serum dilution were prepared on each specimen, 0.2 cc. of packed sheep erythrocytes was added to the one tube, and the same amount of packed human red cells, type O, to the other. These were incubated at 37° C. for thirty minutes with frequent agitation, and centrifuged to separate the cells with adsorbed natural antibodies from the supernatant.

Seven tube dilutions, 1 to 4 through 1 to 256, were run on each specimen, against sensitized sheep and sensitized human, type O, cells; and a serum control of 1 to 4 dilution against unsensitized sheep and human cells was set on each specimen. These tests were incubated in a 37° C. water bath for two hours and preliminary readings were taken. They were then thoroughly shaken and left to stand overnight at room temperature, after which final readings were taken. It was found that only in the most strongly positive specimens were the preliminary readings particularly indicative.

To read the test, the simplest procedure was to place the racks in such a position that they were tilted from the front to at least a 45° angle. By leaving the racks in this position for several minutes before taking the readings, the cells which were not agglutinated would run from a smooth, "button-like" sediment, while

the agglutinated cells exhibited a very typical lace-work pattern. This method of test reading was checked with microscopic readings during the early stages of this investigation with no significant differences noted.

Results

The group of bloods studied included specimens from 123 patients under treatment for clinically active tuberculosis, 127 apparently healthy young adults, and 72 patients under treatment for various non-tuberculous diseases.

The sera of 123 patients under treatment for clinically active tuberculosis, were treated in the method just described. These tests were run by using both sheep and human, type O, erythrocytes; no significant differences in titer were noted in comparing results obtained with the two different species types of erythrocytes. In cases where a difference was shown, it was, in most instances, no more than one tube. Since the higher titers were at times with sheep cells, and at times with human cells, it was considered that these differences could be accounted to normal expected variation.

TUBERCULOUS PATIENTS

Titer	SHEEP RED BLOOD CELLS		HUMAN TYPE "O" CELLS	
	No. Patients	Percentage	No. Patients	Percentage
Neg.	1	0.8	2	1.6
1-4	2	1.6	1	0.8
1-8	11	8.9	8	6.5
1-16	19	15.4	23	18.7
1-32	22	17.9	29	23.6
1-64	20	16.3	17	13.8
1-128	14	11.5	12	9.8
1-256	34	27.6	31	25.2
Total	123		123	

The sera of 127 freshmen medical students were tested in the same way as the group described above. Here again, the differences between results obtained with sheep and human red blood cells were not considered significant. In the four cases which showed

NORMAL HEALTHY INDIVIDUALS

Titer	SHEEP RED BLOOD CELLS		HUMAN TYPE "O" CELLS	
	No. Patients	Percentage	No. Patients	Percentage
Neg.	9	7.1	9	7.1
1-4	25	19.7	19	15.0
1-8	42	33.1	44	34.6
1-16	25	19.7	30	23.6
1-32	9	7.1	15	11.8
1-64	11	8.7	7	5.5
1-128	5	3.9	2	1.6
1-256	1	0.7	1	0.7
Total	127		127	

as much as a two tube difference in titer, the higher titer was with the sheep cells. In each of these cases, the serum control with untreated sheep cells showed a positive reaction, indicating the presence of natural sheep antibodies.

The 72 non-tuberculous patients included 45 sera obtained from the routine serological laboratory at Firmin Desloge Hospital. Of the 45, nine had positive serologic tests for syphilis, and 36 were negative.

One particularly interesting case was included in this series, that of a young man with infectious mononucleosis. The first specimen on this patient showed a 1-16 titer to tuberculin sensitized sheep or human red blood cells. In subsequent specimens, after a notable rise in the level of heterophile antibodies, the sheep cell control became strongly positive. Simultaneuosly, in the test with tuberculin treated sheep cells, the titer of 1-256 was demonstrable. However, in the same specimens, controls with human cells remained negative and no significant change in titer was noted in the test with tuberculin sensitized human erythrocytes.

Of the cases in which a titer of 1-256 was demonstrated, at least two were strongly suspected of tuberculosis, but this diagnosis had not been confirmed.

MISCELLANEOUS PATIENTS
(Non-Tuberculous)

Titer	SHEEP RED BLOOD CELLS		HUMAN TYPE "O" CELLS	
	No. Patients	Percentage	No. Patients	Percentage
Neg.	17	23.7	18	25.0
1-4	15	20.8	15	20.8
1-8	16	22.2	13	18.1
1-16	11	15.3	11	15.3
1-32	4	5.6	4	5.6
1-64	4	5.6	7	9.7
1-128	2	2.8	0	0.0
1-256	3	4.2	4	5.6
Total	72		72	

In the early course of this investigation, an attempt was made to sensitize erythrocytes with purified protein derivative of the tubercule bacillus. A series of 38 high titered sera was run in parallel with the old tuberculin sensitized cells, both sheep and human, and in none of these was the hemagglutinin factor demonstrated.

Discussion and Summary

It was of interest to note that 97.6 per cent of the known cases of tuberculosis tested showed positive hemagglutinins in a titer of 1-8 or higher. Eighty-eight and seven-tenths per cent positives were demonstrated in a titer of 1-16 or higher with

sheep cells, or 91.1 per cent positives with human, type O, erythrocytes. In examining results from the group of known tuberculous sera, it seemed noteworthy that the largest single group, 27.6 per cent with sheep cells and 25.2 per cent with human cells, was that which displayed the strongest reaction, 1-256.

In the normal control series, 79.6 per cent showed titers of 1-16 or below, with sheep cells, and 80.3 per cent with human cells. Of this group, a very large majority was included in those of titer of 1-8 or below.

The figures for the miscellaneous group of non-tuberculous patients seemed to parallel those for the normal control group.

It is possible that in demonstrating this hemagglutinin factor, a measure of the activity of precipitins which react with a carbohydrate fraction of the tubercule bacillus has been made.

Human, type O, erythrocytes were shown to be as sensitive and suitable for this procedure as the sheep erythrocytes. In several cases, where natural sheep antibodies were present in the patients' sera, there was a distinct advantage in the use of human, as compared with sheep cells. The ready availability of this most common of human blood types could also constitute a distinct advantage in a situation where sheep cells were unavailable.

The occurrence of biologically false reactions, both positive and negative, is all too frequent. Until more is known of the exact nature of this test and its limitations, no final conclusions can be drawn.

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CHANGES FOUND IN BLOOD AND HEMATOPOIETIC FUNCTION IN CHRONIC LEAD EXPOSED PAINTERS

By WILHELMINA SCHROEDER, M.T. (ASCP)
506 San Jacinto, Baytown, Texas

If there is an abnormal or excessive exposure to lead, the effects on the hematopoietic system may usually be ascertained by a study of the blood picture.[10] Anemia may result but there is no diminished production of blood, only destructive changes of the circulating cells.[8]

The surfaces of the red cells first swell slightly and then shrink[1] becoming crenated and increasingly brittle, but the interior of the cell is not affected and the hemoglobin remains unaltered. Loss of these red cells is compensated for by erythrocytic regeneration. As the young cells enter the blood stream the destructive influence of lead causes their basophilic substance to coagulate and they can be recognized microscopically as stippled cells.[8] As stated by Aub[1] stippling represents a general pathological reaction of the cell rather than a specific phenomenon attributable to the action of lead.

Conclusions of lead poisoning are often erroneously made from this sign and symptom in as much as stippled cells may often appear in pernicious anemia, leukemia, hemolytic jaundice, poisoning from benzene, aniline or the chlorinated hydrocarbons and other diseases.[2,8] Moreover, stippled cells are not present in all stages of lead poisoning. According to certain authorities, stippled cells usually are present in the acute stage or in exacerbations, as in deleading.[8]

There appears to be no uniformity of opinion as to the effects of lead on the white cells.[8] In clinical reports there is either a mild increase in white blood cells, to about 15,000 per cubic millimeter or a decrease to 4,000 per cubic millimeter.[2] The white count generally appears normal for chronic exposure, but leukocytosis is frequently reported in acute lead poisoning. Few have reported an increase in monocytes, and lymphocytes. Others have found an increase in young granulocytes, even myelocytes, with the consequent increase in the Schilling index (shift to left), frequently with the appearance of toxic granules in the cytoplasm of the neutrophiles.[5,9,12] According to Ferguson and Ferguson,[4] the left shift in the neutrophiles in acute plumbism changes to a right shift in more chronic lead poisoning. The presence of lead in the nuclei of leucocytes was demonstrated by Carles.[3]

This is a report of seventy-six painters performing spray and brush painting and paint scraping on cleaning of dead paint coatings from surfaces for a petroleum refinery. These painters were potentially exposed to lead for periods of at least eighteen months. Because of the duration of exposure, this group was

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studied by clinical laboratory and analytical chemical methods for indications and signs of lead absorption.

Complete blood counts and urinary lead determinations were made. The lead determinations were made in the analytical laboratory of the chemical division under the direction of the industrial hygienist. The urinary lead determination was used as the chief standard and was correlated with an atmospheric lead value. The supervisors, who had little or no paint exposure in their present positions, were used as controls. The average age of the personnel in this group varied from twenty-four to fifty-three years, and exposure periods varied from eighteen months to three hundred and forty-eight months.

CONTROL GROUP

Hemoglobin Percent	Red Blood Count	White Blood Count	Differential	Months of Exposure	Urine Lead Mg./Liter
84	4,500,000	7,450	.....	240	.05
92	4,440,000	10,450	.....	312	None
100	4,600,000	9,250	.....	288	.03
88	4,480,000	6,800	.....	348	.01
98	5,100,000	17,400	.....	264	.04
92	4,700,000	8,200	.....	348	.04
100	5,000,000	11,200	.....	300	.04
100	4,900,000	8,400	.....	348	.02
Average 98	4,718,000	9,906		306	.03

For the purpose of this study the urinary "spot" specimen determination was used, since twenty-four hour urine specimens are impractical to obtain from active workers. The specific gravity was adjusted to 1.024 for the possible dietary effect on the volume excreted. The urinary lead average of 0.10 milligrams per liter of urine and a maximum atmospheric concentration of 1.5 mg. per 10 cubic meters were used as upper limits for completely satisfactory conditions. Rechecks were done on those urine specimens exceeding 0.10 mg/liter.

It was reported by the industrial hygienist[7] that some painters had been exposed to concentrations of dust, originating from cleaning old coating of leaded paints from tank surfaces. Seventeen members of this group (Table I) revealed evidence of lead accumulation with elevated urinary lead excretion. One in this group showed basophilic stippling and toxic granules in the neutrophiles. Three others only showed toxic granules. However, no polychromatic cells or anemia was noted.

The hemoglobin determination for the group was within normal limits with an average of 98.6%. In only one case did the hemoglobin drop to 73% with a red blood cell count of 4,700,000. The red blood cell counts never fell below 4,700,000 per cubic millimeter with an over-all average of 4,920,000. The color index varied from 0.8 to 1.1.

TABLE I

Hemoglobin Percent	Red Blood Count	White Blood Count	Differential	Months of Exposure	Urine Lead Mg./Liter
98	4,900,000	9,250	.....	30	.14
98	4,900,000	6,000	.....	54	.18
96	4,800,000	9,450	Toxic granules	30	.14
92	4,400,000	15,950	.....	42	.15
96	4,680,000	6,200	.....	18	.12
106	.....	8,100	.....	72	.15
100	5,060,000	7,200	Toxic granules	18	.12
92	4,880,000	8,050	.....	18	.17
109	5,290,000	6,000	Toxic granules	18	.14
104	5,050,000	8,350	Toxic granules and basophilic stippling	48	.24
92	4,510,000	13,550	.....	24	.17
89	4,540,000	10,250	.....	54	.12
98	4,850,000	10,500	.....	40	.21
96	5,000,000	6,300	.....	36	.12
98	4,650,000	11,750	.....	3	.13
104	5,490,000	6,800	.....	18	.22
96	4,700,000	9,450	.....	6	.14
Average 97.8	4,856,250	8,538		31	.16

Ten men in this group showed a mild leukocytosis with white blood cell counts over 10,000 per cubic millimeter and one had a count of 17,400, with a period of twenty-two years' exposure; the rest were within normal limits.

All the differentials were stained by the Wright method, and in five cases (Table II) the neutrophils were noted to have large, coarse, irregular, basophilic granules of a deep blue color scattered among the normal pinkish granules. There were but three or four of such neutrophils in the leukocyte count. The monocytes and lymphocytes appeared normal.

One painter with an exposure of forty-eight months showed a urinary lead value of .24 mg/liter, stippled red cells and neutrophils with toxic granules. The hemoglobin in this case was 104%, red blood cell count of 5,050,000, and a white count of 8,350. The man was removed from his job for a short period, and a recheck was made. The urine lead still showed .24 mg/liter, but no stippling or abnormal neutrophils were noted. Of the five men that showed toxic granules in the neutrophils, four of them also show excessive urinary lead excretion and were removed

TABLE II

Hemoglobin Percent	Red Blood Count	White Blood Count	Differential	Months of Exposure	Urine Lead Mg./Liter
104	5,050,000	8,350	Stippling and toxic granules	60	.24
92	4,850,000	9,650	Toxic granules	24	.07
109	5,290,000	6,000	Toxic granules	24	.13
96	4,800,000	9,450	Toxic granules	36	.13
100	5,060,000	7,200	Toxic granules	36	.12
Average 100	5,000,000	8,130		36	.12

from exposure. In about six months, rechecks were made and no abnormal leukocytes were found, and the urine determinations were all either within normal limits or well below the range considered dangerous.

Summary

Seventy-six painters exposed to lead paint products in a petroleum refinery were studied for evidences of plumbism. "Spot" urine lead determinations and complete blood studies were employed to determine abnormal lead accumulation. Of the seventy-six, seventeen showed elevated urine lead concentrations. Five showed toxic granules in the neutrophils. Four of these revealed elevated urine lead determinations, and one urine lead was normal. One showed basophilic stippling with the toxic granules. The seventeen with significant evidence of increased lead absorption were removed from exposure. Recheck determinations were made after a period of six months. In every significant case, the urinary lead concentration and blood picture was found to be improved.

I wish to acknowledge the supervision of Dr. R. W. Pipkin, Assistant Chief Physician, Humble Oil and Refining Company, and the cooperation of Mr. J. W. Hammond, Industrial Hygienist, who initiated this survey and assisted in the preparation of this report.

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COCCIDIOIDOMYCOSIS IN SOUTH TEXAS; CASES OBSERVED IN SANTA ROSA HOSPITAL*†

By FREDERICK W. BIEBERDORF, Ph.D.

Mycologist, Southwest Foundation for Research and Education
San Antonio, Texas

Fungus infections are of such common occurrence that it has become necessary to consider mycotic diseases in the diagnosis of nearly every obscure infection. The emphasis that has been placed on the importance of mycotic diseases can readily be appreciated when one takes note of the large number of papers dealing with the mycoses that have appeared in our scientific literature the past ten years.

While the diagnosis of human diseases is primarily the function of the practicing physicians and pathologists, they in turn rely upon the technologists to assist and frequently verify their conclusions. This dependence upon the diagnostic laboratory then places a serious responsibility upon the laboratory personnel. This means that the medical technologists should have some training or experience in the handling of mycological material. While it is true that certain pulmonary infections caused by fungi are somewhat limited to endemic areas, the movement of both civilian and military personnel in and out of these areas emphasizes the importance of including the mycotic diseases in making any differential diagnosis. The role of the diagnostic laboratory in regard to the mycoses has already been very ably presented by Haley[4] and others and time does not permit its further discussion. Since some of the fungi mimic tuberculosis as well as other diseases, the technologist should then routinely streak out all exudates, sputum or other materials, upon special fungus agar slants.

The recent work by Ajello definitely indicates that the digestion procedures commonly used in the isolation of tubercle bacilli may destroy the fungi, and sputum put through this process cannot be relied upon for the isolation of fungi. Therefore, it is necessary to streak out any sputum or exudate before putting it through a concentration procedure. Ajello and coworkers tested seven pathogenic fungi by putting them through four different concentration procedures and found that most pathogenic fungi do not survive these procedures. This no doubt explains why one sees very few fungus contaminants on Petragnani slants.

Coccidioidomycosis

The etiological agent causing coccidioidomycosis is known as *(Coccidioides immitis)*. This organism has been isolated from soil

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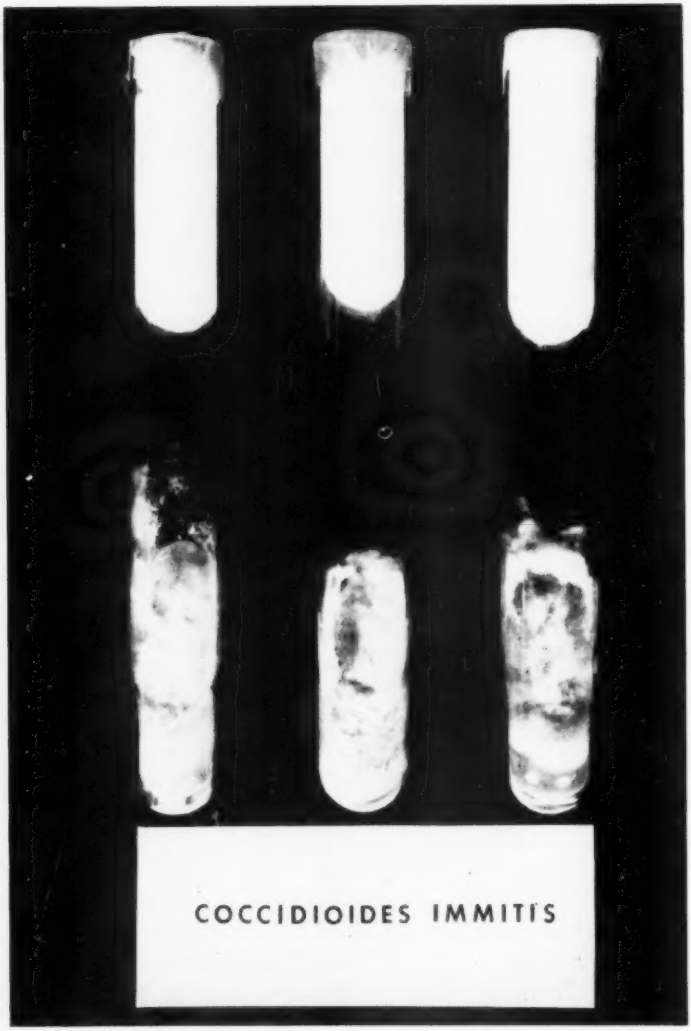
COCCIDIOIDES IMMITIS

by Stewart and Meyer[5] and from rodents by Emmons,[8] and sometimes it also occurs in cattle, sheep and dogs.

Coccidioidomycosis is probably the most infectious of the mycoses and presents several clinical manifestations. The infection may be characterized by presenting a picture of an acute self-limiting respiratory disease or it may progress to the disseminated form, which may involve the viscera, bones, meninges and other tissue.

During the past seven years, over forty cases of suspected *Coccidioidomycosis* have been observed at the Santa Rosa Hospital Laboratory. Twenty of these were definitely proven by the isolation of the organisms. Many of the suspects were single admissions to the out-patient clinic, and four of them were also proven to be tubercular. No attempt was made to follow up these cases. Many of the cases were observed as a direct result of the routine streaking of exudates upon fungus media. Four cases have been selected for presentation.

Case No. 1

S. A., a 50 year old female, apparently well until August, 1951, at which time she began to notice pain in the back of her chest on the left side. Soon after that, she developed a cough that was productive of moderate amounts of greenish-yellow sputum. She also developed a fairly consistant low grade fever. Up to that time, she had been in good health, without any previous operations, serious illnesses, or accidents, and no allergies or asthma. In October, she was admitted to Santa Rosa Hospital and diagnosed as broncho-pneumonia. Her sputum was negative for acid fast bacilli. Her X-rays indicated an extensive pathological process involving both lower lung fields, as well as the right middle lung field.

The concentrated sputum was streaked on sabouraud agar slants. On the first two attempts the results were negative insofar as *(Coccidioides immitis)* was concerned. The only fungi that were observed growing on the slants were common contaminants *Aspergillus* and *Hormodendrum.*

This emphasizes the importance of streaking out the sputum before the concentration process. Apparently the *Coccidioides* was inactivated during the process, and we failed to observe it on culture slants.

The patient was then skin tested with *Blastomycin, Coccidioidin* and *Histoplasmin,* as well as antigens prepared from *Aspergillus* and Hormodendrum. All of the skin tests were negative.

The sputum was streaked out again before the concentration and cultured for one month. The first organism to appear on the slant at 37° C was a yeast growth resembling the yeast phase of *Blastomyces.* After 14 days at 37° C, the slants were then transferred to

R. T., and in another week, developed a white mycelial growth which appeared to be coming from this yeast-like growth. However, on examining some of the mycelium under the microscope, arthrospores could be observed. It required almost a complete month before it was difinitely concluded the organism was *coccidioides immitis* and not *Blastomyces.*

Antibiotic therapy composed of Penicillin, Streptomycin, and Terramycin was administered. While this therapy may at times have appeared to give the patient some relief, it was of short duration. Iodine appeared to have no effect on the progress of the disease. The clinical symptoms appear to be well within the limits of those outlined by Conant.[2]

About four months following the onset of this illness, the patient developed a large abscess on the right shoulder. The abscess was aspirated and about 400 cc of a thick purulent drainage was withdrawn.

This aspirated material was streaked out on a slide and stained with grams stain. Microscopic examination revealed the presence of numerous large thick-walled spherules. This material was streaked on sabouraud maltose agar. Within 7 days, the agar slants were covered with a white cottony mycelial growth at 37° C. The slants incubated at room temperature showed a slightly less growth.

Blood plates streaked with this purulent material showed a white mycelial growth including arthrospore formation in 48 hours at 37° C. The patient became progressively worse and terminated fatally at the end of six months. Autopsy revealed the following organs to be affected: lungs, heart, liver, spleen and kidneys. These organs all contained thick-walled spherules common in the granuloma type of coccidioidomycosis.

Case No. 2.

C. S., a 13 months old male, was admitted to Santa Rosa Hospital with a diagnosis of atypical lobar pneumonia. This patient had a fever for two weeks and a slight cough. After two weeks, the patient improved considerably and the cough subsided. However, the right chest failed to clear satisfactorily; the left lung remained clear.

Culturing the sputum failed to reveal the presence of any fungi. Since the right chest failed to clear, some material was aspirated from this area with a bronchoscope. The aspirated material was streaked on agar slants and a pure culture of *Coccidioides immitis* appeared within 10 days at 37° C. During the period of illness, this patient was placed on an antibiotic therapy, which included Penicillin, Chloromycetin and Terramycin.

Case No. 3.

D. C., an 8 year old male, was admitted to the hospital with the chief complaint of swelling of the left knee of three months duration. Previous to the admission, he developed a sore throat, fever, and occasional vomiting.

Examination revealed the left knee was swollen and tender over the anterior portion of the tibia. X-rays indicated a destructive process of the left tibia involving the epiphysis and metaphysis. Aspiration biopsy of the knee failed to reveal any acid fast bacilli; the tuberculin patch test was also negative. Skin tests were negative for histoplasmin and blastomycin; however, coccidioidin was 1 plus at 48 hours. Chest X-rays showed there was no infiltration of the lung fields.

About six months after the onset of the illness, the patient was taken to the operating room, a curett was inserted and some yellow material was removed. This material on examination revealed the presence of round bodies, characteristic of the spherules found in *coccidioides*.

The wound continued to exude a yellowish liquid which contained the spherules. This exudate was streaked on fungus agar slants, and within a week, a white mycelial growth with arthrospores appeared. Antibiotic therapy composed of penicillin and streptomycin was administered without any apparent change in the patient's condition.

Case No. 4.

D. S., a 25 year old married female, was admitted to the hospital following an illness of approximately 10 days duration. During this period she developed a deep cough productive of a small amount of greenish, very thick sputum, flecked with blood. The fever persisted between 99° and 100.5°. During this period of her illness, she developed an erythemetous muscular rash of both lower extremities which may have been due to some sulfa drugs. The joints in her lower extremities were slightly swollen and painful to the touch and motion. Chest X-rays disclosed a rounded mass in the right middle lobe. A positive skin test was obtained with 0.1 cc coccidioidin using a 1/10,000 dilution. No sputum was sent to the laboratory for culturing and diagnosis was based entirely upon the positive skin test, the Erythema nodosum, and other symptoms common to coccidioidal infection such as low grade fever, chills, and slightly swollen knees and ankles. According to Conant et al.,[2] the progressive type of coccidioidomycosis rarely occurs in patients who develop allergic reactions following the primary reaction.

Conclusion

These four cases of proven coccidioidomycosis emphasize the importance of including the possibility of mycotic infections in

making the final diagnosis in obscure diseases.

They further emphasize the responsibility placed upon the laboratory technician in aiding the physician in reaching the right conclusions.

Three of the cases further verify the findings of other investigators that most of the fungus elements are destroyed or inactivated during the tubercle bacillus concentration procedures. They further indicate that a routine culturing of sputum and other exudates for fungi is highly desirable and should be made before any concentration procedures are done.

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DETERMINATION OF ANTISTREPTOLYSIN TITERS: TECHNIC AND SIGNIFICANCE*

By RUTH ROBINETTE, B.S., M.T. (ASCP)**

Ashland, Kentucky

It is the purpose of this paper to discuss the value of antistreptolysin titer determinations as a laboratory aid in the diagnosis of streptococcal diseases, rheumatic fever, and glomerular nephritis. The principle and technic of the test will also be reviewed.

There is widespread acceptance of the theory that there is a relationship between the Lancefield group A hemolytic streptococcus and the diseases—rheumatic fever and glomerular nephritis. The results of antistreptolysin studies have been used for the past twenty years by workers in the fields of etiology and pathogenesis of rheumatic fever. In more recent years, these determinations have been of accepted value in diagnosis and treatment of the disease.

Yet it is my belief that this test is used almost exclusively in research laboratories, and is not usually included in the armamentarium of hospital and clinical laboratories. The determination can be run by anyone qualified to do bacteriology and immunology; and no special equipment other than that used in the general bacteriology laboratory, is required.

In 1928, Todd[1] first reported the demonstration of a lysin for red cells, or streptolysin—which was liberated by the hemolytic streptococcus. In 1932, he published his method for performing the antistreptolysin test, and his results with that test in patients having streptococcal infections, rheumatic fever, and glomerular nephritis.[2] In 1933, Hodge and Swift[3] published their modification of Todd's technic for determining antistreptolysin titers. Since that time, there have been numerous groups of workers who have published results confirming the concept that there is a significant increase in antistreptolysin titers in patients who have had recent infections by the group A hemolytic streptococcus. Some of the groups have further shown that the titers of patients having rheumatic fever or glomerular nephritis are significantly higher and are more sustained than the titers of patients having uncomplicated streptococcus infections.[4,5]

Except for minor variations introduced by the individual laboratories, workers have used the original technic of Todd, or the Hodge and Swift modification of Todd's technic.

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** Medical Technologist for Dr. Leslie H. Winans, Ashland, Ky., through whose direction and facilities this work was made possible.

The results of antistreptolysin work have been applied to the concepts of etiology of such diseases as rheumatic fever and glomerular nephritis. Evidence has accumulated over the past two decades of a relationship between the group A hemolytic streptococcus and rheumatic fever and glomerular nephritis. It has been proved that these diseases are preceded by an infection by the hemolytic streptococcus, even though that infection may be so mild as to escape clinical detection.[6] A rise in the antistreptolysin O titer is an accepted method of proving streptococcus infections in such cases.

Following the infection, there is a latent period of a few days to three weeks, during which immunological mechanisms can be demonstrated. It has been shown by Todd,[2] Harris and Harris,[7] Quinn,[8] and Rantz,[9] that patients who develop the sequellae of rheumatic fever or glomerular nephritis, develop abnormal amounts of antistreptolysin in their sera, during this latent period.

It has been suggested by numerous workers that the clinical course and the pathological lesions of rheumatic fever and glomerular nephritis could best be explained by an antigen-antibody reaction. Whether some extracellular component of the streptococcus, such as streptolysin O, itself acts as the antigen, or whether the streptococcus product combines with some otherwise non-antigenic tissue protein and forms a hapten-linkage which acts as the antigen, has not been proved.

Extensive studies have been carried out to relate the rise of antistreptolysin titers to the production of autoantibodies to kidney, in the case of glomerular nephritis,[10] and to autoantibodies to connective tissue of the heart and blood vessels, in the case of rheumatic fever.[11] Lange and his group[12] suggest that the presence of high antistreptolysin titers and their increase subsequent to reinfections, seem to indicate that the streptolysins may possibly be the factors which split up certain otherwise non-antigenic tissue proteins, and combine in a hapten linkage so that they become antigenic. In this way, we could have the situation where there is a circulating antibody to the body tissue, and where the reaction of the antibody and the tissue would produce lesions in the kidney, or the heart, or blood vessels, depending upon which type of tissue had become antigenic. This is the autoantibody theory.

Other studies by Rich[13] and Fishel,[14] have indicated that the lesions may be the result of a hypersensitivity reaction between some streptococcus component, such as streptolysin, and the body cells sensitized by an antibody, such as antistreptolysin, which has been fixed to the cells of the host.

But even though the exact antigen-antibody mechanism has not been established, the following facts strongly support the

theory that the pathogenesis of these diseases is on an allergic basis:

1. The history of the percusory streptococcus infection.
2. The latent period following the active infection, during which increasing streptococcic antibody titers can be demonstrated.
3. The accumulated evidence that neither rheumatic fever or glomerular nephritis are themselves infectious diseases.
4. The many features of these diseases which resemble the classical antigen-antibody reaction in serum sickness.
5. The pathological resemblance of the lesions of rheumatic fever and glomerular nephritis to the lesions produced in animals by allergic tissue reactions.

The use of the antistreptolysin test as a diagnostic aid has been stressed by many workers. Reitman[15] suggests that it should be used wherever there is a question of the etiology of infections.

Various groups of workers have presented data to show the diagnostic value of the antistreptolysin test in the following clinical groups:

1. STREPTOCOCCAL DISEASES—In this group a rising titer of the antibody after an infection, is strong presumptive proof of its group A streptococcal etiology.[6] However, it should be stressed that a negative test does not eliminate the possibility of a strep infection. Also, an increased titer in a single serum may be due to either a recent, or an old streptococcal infection. The maximum rise in titer seems to be by the end of the third week after the infection. From the third to the sixth week it remains somewhat stable. After this period, in uncomplicated cases, the titer usually begins to fall, but it may persist for months or even for several years.

2. RHEUMATIC FEVER AND GLOMERULAR NEPHRITIS—The test is also of value in the diagnosis of rheumatic fever and glomerular nephritis. Where the history of a recent streptococcus infection is of importance, it may be particularly helpful to know the antistreptolysin titer. The titer has been shown to be increased in 80 to 90 per cent of the cases in which rheumatic fever develops. Harris[4] and Quinn[5] have found similar increased titers in the case of glomerular nephritis. It has further been found that the increase in titers is greater, and remains so for a longer period of time, in rheumatic fever and glomerular nephritis, than following uncomplicated streptococcal infections. According to Harris[4] the similarity of titers in rheumatic fever and glomerular nephritis has not affected the limited usefulness of the test toward laboratory diagnosis since they do not offer a differential problem. This test has been recommended as an *aid* in the differential diagnosis of these two diseases from other diseases. But all groups of workers have cautioned that it is not a diagnostic test in itself, and that there are many limiting factors to be considered in using it. Only if these limitations

are recognized can it be used to the best advantage. In our own laboratory this test has been particularly helpful where there were increased titers on patients who had clinical signs of rheumatic fever; but with whom there was no history of a recognized streptococcus infection. We have also found increasing titers helpful in judging when quiescent cases of rheumatic fever or glomerular nephritis are becoming reactivated. Considered with other clinical and laboratory data; we have felt that the test is of practical value as a diagnostic aid.

Antistreptolysin testing has further been applied in methods of prevention and treatment of streptococcal diseases, rheumatic fever, and glomerular nephritis. Kilbourne and Loge,[16] Weinstein,[17] and Rammelkamp[18] have shown that penicillin therapy of streptococcus infections suppresses the formation of antistreptolysin. Rammelkamp theorized a method of prevention of acute rheumatic fever in the following manner: He used the established fact of an elevated antistreptolysin titer in the sera of patients with acute rheumatic fever. He noted further that the antibody titer of sera taken during the latent period following a streptococcal infection is higher in patients who develop rheumatic fever, than it is in those who do not develop this disease. Since the attack rate of rheumatic fever in any population with acute streptococcal infections can be correlated with the rise in antibody response as measured by the antistreptolysin titer, he then concluded that the ideal form of preventative therapy would be that which inhibited antibody production to the maximum degree. From this line of reasoning, Rammelkamp conducted experiments and presented data[19] indicating that adequate penicillin therapy of acute streptococcal infections almost completely prevents a subsequent occurrence of acute rheumatic fever.

Having discussed the development and applications of the antistreptolysin test; I should like to review briefly the immunological principle involved. The group A hemolytic streptococci produce a toxin, streptolysin, which hemolyzes red cells. This toxin, streptolysin, will also act as an antigen and will cause an antibody to be formed. This antibody is antistreptolysin. The antistreptolysin can combine with the streptolysin and block it, so that the streptolysin can no longer hemolyze red cells. A test for the amount of antistreptolysin which has been formed by an individual, has been devised. This test is reported as an antistreptolysin titer. Various dilutions of a serum containing an unknown amount of antistreptolysin are made. These dilutions are added to known amounts of the streptolysin, expressed in units. An indicator system of a suspension of red cells is added to the mixture. If the streptolysin is not blocked by an antistreptolysin in the unknown serum, it will cause hemolysis of the red cell suspension. If there is an antistreptolysin content to

the unknown serum, the hemolysis will be inhibited. The higher the amount of antistreptolysin in the unknown serum, the higher it can be diluted and still contain sufficient antistreptolysin to inhibit hemolysis. The titer of the test is therefore, read as the highest dilution of the unknown serum which will completely inhibit hemolysis by the known amount of streptolysin, in an indicator system of red cells. It has been found that there is a relationship between certain ranges of antistreptolysin titers and various diseases related to streptococcus infections.

As I have indicated previously, there are several modifications and variations of Todd's original method. The technic which I shall describe is a variation of the modification of Hodge & Swift.[3]

1. Serum is inactivated at 56 degrees centigrade, for 30 minutes.
2. Make serial dilutions of the serum in physiological saline, with 1.0 ml. volumes. The initial dilution is 1:50, and proceeds in decrements of 50 to 1:500, i.e., 1:50, 1:100, 1:150, 1:200, etc.
3. Use 10 tubes for each serum tested. Each tube contains a 1.0 ml. volume of serum dilution.
4. Standardized streptolysin is diluted with saline so that 0.5 ml. represents one combining unit as previously determined in the standardization of antigen. Add 0.5 ml. of the diluted antigen to each tube. Shake.
5. Let stand at room temperature for 15 minutes.
6. Add 0.5 ml. of a 10% suspension of washed red cells to each tube. (Human O or sheep cells—if rabbit cells are used, use a 5% suspension.) Shake.
7. Incubate at 37 degrees centigrade for exactly 60 minutes.
8. Allow the cells to settle and read. If the tests are left overnight for reading, they should be refrigerated.
9. The last tube showing no hemolysis represents the number of units of antistreptolysin in the serum tested.
10. Report the titer as the number of antistreptolysin units. This would be the reciprocal of the fraction of a cubic centimeter of serum contained in the highest dilution showing no hemolysis. For instance, if the last tube giving an O reading is a 1:200 dilution, then the titer is recorded as 200 antistreptolysin units.
11. Run sera of known titrations and a hemolysin titration check with each group of tests.

The following schedule is used for reading the test, and one of the following symbols is recorded for each tube:

O is for no hemolysis, a clear colorless supernatant, and a button of cells in the bottom of the tube.

tr is for a very faintly colored supernatant, well settled, large button of cells in the bottom. This is counted as no hemolysis.

w is for a weak hemolysis with weakly colored supernatant, and a slightly decreased button. This counts as one-half in the titer dilution.

s is for strong hemolysis, red supernatant, a small button of cells. This is counted as completely hemolyzed.

ac is for almost complete hemolysis, red supernatant, a few cells in the bottom. This is counted as completely hemolyzed.

c is for complete hemolysis, clear red supernatant, no cells left.

The reading of the antistreptolysin titer is for the last tube giving complete *inhibition* of hemolysis, or for the last O reading. In contrast, the reading of the hemolysin titration is for the last tube showing complete hemolysis, or for the last s to c reading.

Reagents to be used in the test include:

SERUM: The serum should be refrigerated if not run within 24 hours. Streptolysin is quite stable and will remain indefinitely if kept frozen.

STREPTOLYSIN: So far as I have been able to ascertain, this antigen is not commercially available. It can be prepared by the following method: Three liters of dextrose broth (Difco), in an Erlenmeyer flask, is seeded with 300 cc of a six hour culture of a group A, hemolytic streptococcus, such as the NY5 strain, which is a good streptolysin producer. After an eighteen hour incubation period, the culture media is freed of organisms by filtering or centrifuging. The supernatant, in 200-300 cc aliquots, is poured into sausage casing and preevaporated in front of an electric fan, to approximately one-fifth its original volume. This gives a very concentrated supernatant which has a high salt content. This is recentrifuged at 5000 rpm for 30 minutes. The clear supernatant is transferred to another, smaller sized sausage casing, and is dialyzed in running tap water for 24 hours. This filtrate, which represents streptolysin in its inactive form, is then reduced in vacuo to the active, or hemolytic, streptolysin O. This is accomplished by adding 5 grams of sodium bisulfite per liter and maintaining a vacuum until only a few bubbles are given off. The final reaction after complete reduction must be slightly alkaline. The streptolysin is then tubed in small portions, sealed with warm, sterile vaseline, and refrigerated. Kept in this manner, the streptolysin is stable for a long period of time.

Some groups prefer to prepare a supernatant that is even more highly concentrated than described above. Such a supernatant is then transferred to wide mouth bottles, frozen by rotating in dry ice and water, and dessicated by suction. The dry powder is stable indefinitely. It must be diluted with saline, and reduced by addition of sodium bisulfite, just prior to setting up the test.

STANDARDIZATION OF STREPTOLYSIN: The streptolysin must be standardized so that 0.5 cc of the antigen used in the test procedure will contain one combining unit or 2½ minimal hemolytic doses. Todd standardized his antigen by determining the minimal hemolytic dose, hereafter referred to as mhd. One mhd is the amount of hemolytic broth required to hemolyze completely 0.5 cc of 5% rabbit blood cells in one hour. The hemolytic titration is run by the following method: Beginning with 0.5 ml. of hemolytic broth, decreasing quantities in steps of 10% are placed in a series of 10 tubes. Saline is added to make a 1.0 ml. total volume of each tube. Add 0.5 ml. of a 5% suspension of washed rabbit cells to each tube. Shake and incubate for 60 minutes. The last tube showing complete hemolysis contains 1 mhd. Todd then defined one antistreptolysin unit in a given serum, as the amount that would neutralize 2½ mhd of streptolysin. One unit of streptolysin was set as the amount which would be neutralized by one unit of antistreptolysin. So one unit of streptolysin would correspond to 2½ minimal hemolytic doses.

Hodge and Swift, however, felt that Todd's minimal hemolytic dose in a given streptolysin would vary, whereas the combining power with antistreptolysin would remain stable. They stated that it was essential to know the combining capacity of streptolysin, rather than only its exact hemolytic power in terms of mhd. They defined one streptolysin unit as the least amount of broth showing no hemolysis when combined under test conditions, with a standardized serum representing one unit of antistreptolysin.

Todd suggested that a serum containing a known number of antistreptolysin units be universally employed as a standard. No such standard serum is really available, so in order to keep the same system of units employed by Todd, workers have standardized their streptolysin with sera which have been duplicated by standardization by Tood or his followers. This means that when a batch of streptolysin antigen is made, it should be standardized by a titration of combining power—with some previously standardized serum. The titration of combining power is carried out in the following way: In a series of ten tubes, the streptolysin which is to be standardized, is diluted by 10% decrements. The volume in each tube is made 0.5 ml. with saline. To each tube, a 1.0 ml. dilution of a known standard serum is added—the serum having been diluted so that 1.0 ml. contains 1 antistreptolysin unit. Shake and incubate at 37 degrees centigrade for 15 minutes. Add 0.5 ml. of a 5% suspension of rabbit blood cells to each tube. Incubate for 60 minutes, let cells settle, and read results. The amount of broth in the last tube showing no hemolysis represents one unit of streptolysin. The streptolysin antigen to be used in titrating unknown sera must then be diluted with saline, so as to contain one unit per 0.5 ml.

I don't think it matters which particular variation of the original method that is used for the antistreptolysin determination. The principle of all the technics is the same. I have reviewed the one with which I am the most familiar. If technics were perfectly standardized, and the same one was used by all groups, it would be easier to compare results from one laboratory to the other. However, the clinical significance of the test lies in comparison of mean titers of the normal groups and various disease groups. As a number of the tests are run, an evaluation of titer variations is made possible.

As the test becomes more widely used, progress should be made towards standardization of technic and evaluation of results. With wider use, also, perhaps commercial sources of reagents will become available, thus eliminating the time-consuming part of the test in preparing antigens.

Summary

In summary, this paper has pointed out the values and significance of the antistrepotolysin test as a laboratory procedure, and has described a technic for performing the test.

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"EXPERIENCE WITH A BONE BANK OPERATED AT A VERETANS ADMINISTRATION HOSPITAL"*

By NORMA J. CAMBRON, M.T. (ASCP)**

I propose, in this paper, to illustrate how bone is procured, prepared and stored in the Bone Bank of the Veterans Administration Hospital, Louisville, Kentucky. The method is essentially similar to that described by Gordon and Welsh.***[1]

Our main source of bone is from amputated extremities of patients with gangrene resulting from arteriosclerosis or diabetes. When we are notified that a leg, suitable as a source for bone grafts, is to be amputated at a local hospital, we send a messenger by automobile and have the leg brought to the laboratory, in sterile sheets and protected by paper wrappings. The portions of bone which are to be used are removed in the operating room by one of our doctors. He prepares himself exactly as he would for a major operation, and removes and strips the bone aseptically.

After removing the bone and cutting it into strips varying in size from 15 to 20 cm. in length and 2 to 3 cm. in width, the soft tissues, including as much of the periosteum as possible, are stripped from the surface. The fat is scraped away from the marrow cavity. The strips are then placed in 1 liter size beakers, hydrometer jars, or plain cylinders. The hydrometer jars† are most satisfactory because they do not need a large volume of fluid or much storage space in the refrigerator yet provide adequate volume for the bacteriostatic fluid in which the bone is to be stored. With the smaller volume of fluid in the hydrometer jars it is easier to rule out contamination than with the beakers or plain cylinders where there is a higher dilution. These hydrometer jars are 25 cm. tall and 3½ cm. in diameter. They may be covered with gauze and protected with heavy brown paper fastened with a rubber band. (Only one accession is placed in each cylinder, in order that no more than one accession need be discarded, should the solutions become contaminated.) The glass containers are filled with sterile saline. (Care should be taken not to fill the jar so full that the gauze becomes moistened when

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** From the Laboratory Service, Division of Bacteriology and Serology, Veterans Administration Hospital, Louisville, Kentucky.

Reviewed at the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author is the result of her own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

*** Other methods of preserving and "banking" bone, are listed at the end of this paper. See References 2 through 7.

†May be procured from A. S. Aloe, 1831 Olive Street, St. Louis 3, Missouri.

the jar is moved.) Each cylinder is marked with a special identification tag listing the following data: name of donor, hospital from which received, date received and the nature and source of the graft. The tag is also stamped "NOT READY FOR USE." The bone is then ready for initial storage. It is stored in a mechanical refrigerator at about 39° to 41° F.

All of the small pieces and scraps are saved. These are used to make bone meal or chips which the surgeon uses to pack around the grafts. The meal is ground in a bone mill while the graft is being inserted.

After 5 days' storage, the grafts are placed in a fresh solution of saline and the initial solution is cultured aerobically and anaerobically, using 0.5 cc. of the saline in each of 5 tubes of thioglycollate medium. (The use of 5 separate culture tubes permits an additional safeguard for eliminating contamination. It has been our experience, in a few rare instances, that bacterial growth may be demonstrable in only one of the five tubes; therefore we use as many tubes as practicable.) The saline in which the grafts are stored is changed three times, at intervals of five days between each, the fluid being cultured after it has been used for storage. After the third saline, the grafts are placed in 1:1,000 aqueous solution of merthiolate. This is cultured after the bone has been stored in it for five days. The solution is changed to 1:6,000 aqueous solution of merthiolate. The grafts remain in the 1:6,000 aqueous solution of merthiolate until used; however, this solution is cultured after five days.

When the final change has been completed and no evidence of contamination has been demonstrated, the accession identification tag is changed to read: "READY FOR USE NO....." Three accession sheets are made out for each piece of bone and each piece is given an individual number, in numerical sequence. The top half of the accession sheet has the following information: accession number, donor's name, hospital, type of bone, date bone was received, date solutions were changed, and the date and result of the cultures. The bottom half of the slip is for the clinical data, to be filled out by the surgeon, listing the name of the recipient, the hospital, the type and location of the graft, diagnosis, date grafted, amount used, and the result. Two of the slips are given out with the bone and the other is kept by the laboratory. Of the two given to the doctor, one is returned to us and the other is placed in the patient's chart. The third copy is permanently filed, after the information from the returned copy has been typed in. An accession record book is also kept.

The method of grafting is done according to the experience of the surgeon, the nature of the disease process which is to be corrected, and the character of the graft. Most of the grafts are

of the so-called "strut" type, reinforced with "bone chips". These are inserted as a source of support for a weak or defective region in the spine or one of the extremities of the recipient.

When the surgeon does not use the entire piece of bone, the unused portion is returned to the laboratory. It is recultured, given a new number and accession sheets, so that it can be used as an additional source for grafting.

"From a little acorn a mighty oak doth grow" might aptly be applied to the Bone Bank. In July 1950, when we started keeping permanent records of accessions and withdrawals from our Bone Bank, an average of 1 to 4 pieces of bone was used a month. Now the average is 25 to 30 pieces a month, and the demand is becoming greater all the time.

Summary

Our method for procuring, preparing and storing bone, for use as homologous grafts, has been described. The method is simple, utilizing equipment which is inexpensive and readily available. The use of hydrometer jars permits storing of many accessions on a single shelf of a mechanical refrigerator. It facilitates detection of bacterial contaminants because only a small amount of storage fluid need be used. The jars are tall enough and wide enough to accommodate accessions large enough for almost any type of bone grafting procedure.

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A METHOD FOR RECORDING LABORATORY PROCEDURES

By ALICE T. STROZIK, M.T. (ASCP)
St. Joseph's Hospital, Syracuse 3, New York
and DORIS I. CHAPPELL, Medical Secretary
St. Joseph's Hospital, Syracuse 3, New York

Due to the rapidly increasing uses of micro-photography in the present-day hospitals, a suitable method for recording Laboratory procedures is essential. A single sheet on which all the Laboratory procedures are listed and the findings recorded thereon, is unsuitable for micro-filming; therefore, a method utilizing the least number of sheets and still maintaining a comparative study for the physician, is the most desirable. Such a method is presented here, having the added advantage of keeping clerical work to a minimum and effecting the minimum amount of change of forms in a hospital.

The chart form consists of separate sheets for Chemistry, Hematology and Urinalyses. The latter also includes a space for Kidney function tests, Gastric Analysis and Stool examinations. These sheets have the various tests listed vertically giving the normal range in parentheses after each test. Listed horizontally are spaces for the dates. There is sufficient space for a period of nine days which is two days longer than the average hospital stay of patients at St. Joseph's Hospital. The form also includes a sheet for Bacteriology.

It has been necessary to prepare a similar short form for use in the minor surgical cases such as: T. & A.'s, D. & C.'s, etc. This consists of one sheet which includes only the pre-operative tests usually requested for these procedures such as: CBC, Bleeding and Clotting Time, Sedimentation Rate and Urinalysis.

Reports are recorded on the request forms (size 5½" x 7¼") which are delivered to the Laboratory. These request forms are used as work sheets. When the test is completed, it is noted on the daily charge sheet, which also serves as a daily statistical tabulator. The request forms (work sheets) are then taken to the floor, recorded on the form in the patient's chart and then brought back to the laboratory where they are filed alphabetically. This method of filing is advantageous to the laboratory as all of the reports of each patient are together.

Master sheets of the chart forms are made here with a print-type typewriter and copies are made on a multigraph machine on Bond paper. For best results in micro-filming, the results of the examinations are recorded with black ink.

Summary

A method for recording laboratory procedures is presented which: 1) is suitable for micro-filming, 2) gives the physician the opportunity to compare results at a glance, 3) does not involve complicated clerical work and 4) is economical to establish.

THE GAVEL

I hold in my hands the symbol of our maturity as a Society, The Gavel; a cherished gift because it was presented to us in 1950 by the Past-Presidents of the American Society of Medical Technologists. The donors felt that we had matured enough to merit such a gift. Those who have served as our Presidents have served with dignity and integrity. It is my wish that I may prove worthy of following in their footsteps.

In accepting this GAVEL I am inspired by the beautiful inscription engraved thereon: "With ever increasing strength and humility, and with pride and integrity in our profession—Let Us Serve."

I accept this office with humility—humility because of the wonderful work done by those who have served before me; humility because of the confidence that you have shown in electing me to this position of responsibility. I accept this Gavel with pride—pride in my profession, pride in my professional society, and pride in my fellow-workers. I accept this Gavel with confidence—confidence in the Board of Directors, confidence in the committee members, and confidence in you. We are dependent upon your help and co-operation. We are confident that you will give it. I accept this Gavel with a sense of responsibility—responsibility for the conduct of this office for 1952-53. I am fortunate in having a fine Board of Directors, elected by you, to share in this responsibility. I am fortunate in having committees composed of enthusiastic members—members willing to work for the progress of our society, members interested in the future of medical technology. They also share in the responsibility for the conduct of the affairs of this society whether acting under a directive from the Board of Directors, or carrying out a project assigned by the House of Delegates. I accept this Gavel as a challenge—a challenge to assure ASMT of continued progress and greater achievement.

In accepting this Gavel, I want to assure each of you that the Board of Directors will conduct the business of this society to the best interest of THIS SOCIETY. Your wishes as expressed by the House of Delegates will be carried out. This is your society. It is up to you to say what you want done. With your help, we can truly serve with integrity.

I do not feel that I can close this first message to you without telling you how proud I am of the committees. Each new member on the committee wrote a very enthusiastic acceptance. Each felt that it was an honor to be asked to serve. Each expressed his willingness to help in the continued growth and achievements of ASMT. It was my hope that we could have every affiliate society represented by having at least one member on a committee. Alas, this could not be accomplished for various good reasons. Try setting up ten committees and you will find out what I mean. Each member is a joy to know, and I am glad that I have had this experience—even if only by mail.

I am sure that each of you has the same feeling of loyalty and devotion. With such a feeling we cannot help but have a progressive and bright future.

Sadie Cartwright

AMONG THE NEW BOOKS

LABORATORY MANUAL FOR GENERAL BACTERIOLOGY. 4th edition. Compiled by George L. Peltier, Professor of Bacteriology, Carl E. Georgi, Professor of Bacteriology and Lawrence F. Lindgren, Instructor in Bacteriology and Pathology, University of Nebraska, Lincoln, Nebraska. 293 pp., John Wiley & Sons, Inc., New York, Chapman & Hall, Limited, London. Price $3.50.

This is the fourth revision of a teaching manual which has been improved and brought up to date by the authors to meet specific needs of students in the study of microbiology. It's contents consist of: (1) Introduction, (2) Preliminary Survey of Microbiology, (3) Morphological Structure and Chemical Composition of Bacteria, (4) Pure Culture Technique, (5) Metabolism and Products of Microbial Growth, (6) Factors Influencing the Growth and Death of Micro-organisms, (7) Applied Phases of Microbiology, (8) Determinative Bacteriology and (9) Appendices. It contains sixty exercises with a blank page between each for student's notes or illustrations, each exercise consisting of: (1) Purpose, (2) Materials, (3) Procedure, (4) Record of Results, (5) Questions, and (6) References.

The material is excellent for a first course in bacteriology, but it could also have a place in the library of a hospital laboratory having a school for medical technologists.

MARION TULLY

DIAGNOSTIC BACTERIOLOGY, 4th edition, by Isabelle Gilbert Schaub, A. B. Technical Director, Clinical Bacteriology Laboratories, The Johns Hopkins Hospital; Instructor in Bacteriology, The Johns Hopkins University School of Medicine and the Nurses Training Schools, The Johns Hopkins Hospital and Sinai Hospital, and M. Kathleen Foley, M. A. Instructor in Bacteriology, Department of Biological Sciences, College of Notre Dame of Maryland; Formerly Bacteriologist in Charge of the Diagnostic Bacteriological Laboratories of the Medical Clinic, The Johns Hopkins Hospital. pp. 341. The C. V. Mosby Company, St. Louis, 1952. $4.50.

As the authors state, this is a textbook for the isolation and identification of pathogenic bacteria, which contains no illustrations or plates. It is a small, very compact, modernized textbook which differs from textbooks of general bacteriology. It is well organized, being divided into five parts. Part I—Bacteriological Methods, Part II—Bacteriological Diagnosis, Part III—Determination of the sensitivity of bacteria to antibiotics, Part IV—Serologic Diagnosis, and Part V—Media, Stains and Staining Technique, Reagents, and Tests.

The recent years have brought many changes in diagnostic bacteriology and these authors have given us a book of definite methods which are not always covered elsewhere.

This fourth edition of Diagnostic Bacteriology would afford a good guide and reference book in hospital schools for medical technologists as well as for clinical bacteriologists.

MARION TULLY

SPACIAL VECTORCARDIOGRAPHY, by Arthur Grishman, M. D., Adjunct Physician for Cardiology, the Mount Sinai Hospital, New York; Adjunct Physician in Medicine and Assistant Roentgenologist, Beth Israel Hospital, New York; and Leonard Scherlis, M.D., Research Assistant in Cardiology, The Mount Sinai Hospital, New York. 217 pages with Figs. XII, 5. Philadelphia and London: W. B. Saunders Company, 1952. Price $6.00.

In 1948, investigations in spacial vectorcardiography were started at the Mount Sinai Hospital in New York. The authors of this book do not attempt to discuss all the numerous problems involved in this subject, but aim to give only on over-all view of the field.

Electrocardiography provides an accurate and complete picture of the electrical activity of the heart, the aim of which is to provide some means of arriving at a precise diagnosis. For this, multiple leads must be utilized, some of the technics of which are of limited value. Spacial vectorcardiography, on the other hand, secures quickly and routinely a very complete

representation of the electrical activity of the heart, with which the routine and specialized leads can be correlated and may even eliminate the necessity of their use.

Each lead is treated separately in the conventional concept of electrocardiography, and the patterns recorded by unipolar leads are considered as unrelated records, and are assumed to reflect the electrical events taking place in the heart muscle immediately under the electrode, so that multiple leads are necessary if more information is desired as to the electrical activity of the heart. In the vector concept, however, the leads are treated as derivatives of a single unit, the "spacial cardiac vector," and thus all information concerning the electrical activity of the heart can be presented as a single curve, the "spacial vectorcardiogram," from which the configuration of standard and non-standard leads can be predicted.

This book attempts to present those features of spacial vectorcardiography which make it a scientific basis for instruction in electorcardiography. Medical technologists will find the chapters dealing with the theoretical and technical discussions of great value to them. Several chapters deal with the correlation of scalar electrocardiography and spacial vectorcardiography, and the remaining chapters take up separately all the various phases of cardiographic interest. The extensive bibliography provides a great volume of material for further study of the subject.

ESTHER LEMONT

The Clinical Significance of Increased Rouleau Formation in Smears of the Peripheral Blood, Edwin D. Bayrd, M. D. Div. Med., Mayo Clinic, Rochester, Minn., Am. Jr. Clin. Path. 21:777-783. Aug. 1951.

This author points out that although the importance of rouleau formation in smears of peripheral blood has long been noticed, other clinical aspects of its occurrence have been generally unappreciated.

414 cases of excessive rouleau were reviewed at the Mayo Clinic and then correlated with the diagnosis, total serum, protein and alubumin-globulin ratio. The following rouleau grading was used in studying these cases.

Grade 1 Short chains. Numbers of erythrocytes still not involved.
Grade 2 Chains longer than Grade 1, often broken and slightly retracted. Greater affinity of erythrocytes for each other.
Grade 3 Appreciable extension of the phenomena noted in Grade 2.
Grade 4 Very compact cell groups amounting at times to clumps rather than chains.

A good illustration of a normal blood smear is provided with which the four grades can be compared. Tables are also given showing the degree of rouleau formation in persons with significant organic diseases and miscellaneous conditions. There is another analysis of changes in blood and of disease associated with rouleau formation, one giving albumin-globulin ratios, and two showing the proportion of patients with rouleau formation having significant organic disease in Grades 1 & 2 and Grades 3 & 4. All smears graded No. 4 were given further study for evidence of myeloid immaturity, monocytosis, lymphocytosis, macrocytosis, myeloma plasma cells and apparent increase or decrease in platelets. The case histories presented showed that detection of excessive rouleau formation facilitated the diagnosis, although the diagnosis was not dependent on it.

The 414 cases studied showed the sedimentation rate was roughly proportional to the degree of rouleau formation. Protein changes were less consistent. The alubumin-globulin ratio remained within normal range in more than one-fourth of the cases. 93% of the patients with excessive rouleau formation had significant organic disease falling into the following classifications, 36% neoplasms, 17% infections, 9% hepatic diseases, 9% collagen disease, 6% renal disease, more than a fifth fell into an unclassifiable group consisting of indeterminate anemias, presumptive malignant lesions, myxedema, skin disease, etc. There was evidence for underlying neoplasm but against leukemia if there was simultaneous occurrence of myeloid immaturity and excessive rouleau formation. Hepatic disease was present when macrocytosis was associated with high grade rouleau formation.

ESTHER M. LEMONT

Infectious Mononucleosis: the Prognostic Significance of Various Changes of the Blood Leukocytes, John E. Stevens, M.D., Edwin D. Bayrd, M.D., Frank J. Heck, M.D. Div. of Med., Mayo Foundation & Mayo Clinic, Rochester Minn., Blood. The Journal of Hematology. 7 (No. 1): 31-37, Jan. 1952.

In studying peripheral blood smears in selected cases of infectious mononucleosis, it was found that the clinical course was likely to be severe when toxic changes were present in the granulocytes. Type III lymphocytes and granulocytic immaturity were frequently seen when the onset was acute, but the predominance of any of the various types of lymphocytes of infectious mononucleosis bore no relationship to the clinical course.

The disease received its name "infectious nononucleosis" in 1920, at which time there was a significant step forward in the knowledge of the disease in the recognition of characteristic changes of the abnormal cells in the peripheral blood. The abnormal cells were noted early in 1920 and distinguished from those seen in leukemia. By 1923 the cells were shown to be mature and well differentiated. Heretofore, many observers had

termed them "monocytes", others classified them as immature lymphocytes. In a study made at the Mayo Clinic of 210 sporadic cases from 1937-1947, 96.6 per cent of the smears showed a significant increase in a typical lymphocytes characteristic of infectious mononucleosis. Half of the cases showed these changes were noted the eighth day of illness, but the average time was fourteen days.

To ascertain the prognostic significance of leukocytes, 25 cases were studied to get the relationship of the onset and clinical course to the cytological changes of the leukocytes.
The cases were divided into four groups.

1. Acute onset, severe clinical course, 7 cases
2. Acute onset, mild clinical course, 6 cases
3. Insidious onset, severe clinical course, 6 cases
4. Insidious onset, mild clinical course, 6 cases

With negligible symptoms, absence of fever or low grade fevers, and duration not unduly protracted, the clinical course was considered mild. The course was considered severe with pronounced protracted systemic reaction, high fever, extensive pharyngitis, and adenitis. The group selected had either a positive blood smear or positive heterophil aggulutination test or both in addition to compatible clinical findings. With a titer of 1:112, the heterophil agglutination test was considered suggestive. 1:224 was considered positive. When 50% or more lymphocytes appeared in the differential, half being a typical of infectious mononucleosis, the smear was considered positive.

The careful study of morphologic features of leukocytes included

1. Differential count
2. Differential count of 100 lymphocytes noting ratio of normal lymphocytes to those atypical of infectious mononucleosis, and relative proportion in latter of each of three types.
3. Search for granulocytic immaturity
4. Observation of toxic changes in granulocytes
5. Shift to left of neutrophils

Twenty-five patients were studied. Fourteen had type I lymphocytes predominating, ten had type II, and one had type III. This last one was originally diagnosed as leukemia. Of sixteen patients with some type III lymphocytes, eleven had an acute onset. However, the mild course was as frequent as the severe irrespective of cells. Six cases showed granulocytic immaturity. A moderate shift to the left was observed, but was not constant. Only three showed significant "toxic" changes in granulocytes. Following is the differentiation of the three types.

Type I—Nucleous indented or lobulated with coarse chromatin mesh, abundant, usually deeply basophilic and vacuolated cytoplasm.

Type II—Nuclei generally centrally situated and coarser than type I. Cytoplasm more abundant and appears almost a frill around nucleous.

Type III—Nucleous larger, contains nucleoli, is leukemic in character, Cytoplasm retains most of features of type I.

In this study, type III were encountered in 16 of 25 cases, but only preponderant in one, while previous workers noted them but rarely or when present, not very numerous. 69% of patients having type III were among those with acute onset, so this suggests a relationship between the type of onset and occurrence of these cells. The number of cases, however, is too small to be more than suggestive.

The Effect of Smoking on Hyperthyroidism. Elmer C. Bartels, M.D., Dept. Int. Med. Lahey Clinic, Boston, Mass., James J. Coll, M.D., Duluth Clinic, Duluth, Minn., The Lahey Clinic Bulletin, Boston, Mass., 7:(No. 6) 167-172, Oct. 1951.

From observation of hyperthyroid patients at the Lahey Clinic during a one to six month pre-operative preparation period, there was sufficient evidence to warrant prohibiting of smoking.

For many years the Lahey Clinic had an established custom of advising patients with hyperthyroidism, to abstain from smoking during the pre-operative and post-operative period. In addition to its general effect of intoxication, it was Dr. Lahey's clinical impression that smoking was harmful as it seemed to accelerate the pulse rate.

With the advent of new anti-thyroid drugs and after sufficient experience was gained by their use, it became possible to predict with fair accuracy, the return of the basal metabolic rate to normal. Failure to stop smoking was the basis for failure to achieve the anticipated subjective and objective improvements. In the general population, two out of every three men (66%) and two out of every five women (40%) smoke. The percentage among the hyperthyroids is 73% of men and 56% of women.

Changes in the metabolic rate of normal individuals following smoking one or two cigarettes have been reported. The change usually was not great, averaging about plus 3 for the entire group. The rise was noted in five to ten minutes after smoking, with a lesser secondary rise thirty to forty minutes later. There was less effect with habitual smokers than with occasional ones. As there had been no reports on the effect of smoking on the metabolic rate of hyperthyroid patients, this was investigated by studying moderately severe hyperthyroid patients. Two consecutive tests were done, then the patient remained at bed rest, smoked two cigarettes, and then two more consecutive rates were recorded. Six of these patients had a similar study following a period of anti-thyroid treatment when they had reached an euthyroid state. This permitted comparison in the same patient when hyperthyroid and when euthyroid.

Twenty patients were tested, thirteen (62%) showed a rise in the metabolic rate, the increase varying from plus 3.5 to 23 with an average of plus 8. Five patients (24%) showed no change and three showed a drop of 9, 10, and 3. 52% were observed to have an elevation of blood pressure and 48% had a constant level, the average rise being 15mm. 75% had an increase in pulse rate ranging from 8 to 30. Only five patients showed a rise in all factors, so there was no parallel change in the basal metabolic rate, pulse and blood pressure in the individual case. However, the pulse rate usually rose when the basal rate increased. Five patients were studied while hyperthyroid and aagin when euthyroid and revealed no essential change in comparison of basal rate, pulse and blood pressure. Patients continuing to smoke failed to show a favorable gain in weight following anti-thyroid treatment.

These studies show there is not an exaggerated degree of response of the basal metabolic rate and blood pressure to smoking in hyprthyroid patients, and although the response of the rate in them is no diffeernt than in normal individuals, this does not mean that smoking is harmless to the thyrotoxic patient, since 62% who have an increase in the metabolism are made to suffer from hypermetabolism at a high level. The added burden of this increased metabolic rate is obvious in those patients with initial high levels. Hyperthyroidism does not change the sensitivity to tobacco, but sufficient evidence was exhibited to warrant the prohibition of smoking.

(The fractioning curves of serum proteins with sodium thiosulphate in normal and pathological human sera.) J. Gras.* *R. esp. Fisiol.* 7. 265-280.

The fractioning curves of serum with sodium thiosulphate, a salt suggested the first time in an earlier paper, are studied. From the average curves of a series of 12 and another of 35 sera the existence of three main points of inflection is established at the concentrations of 30,7, 35,5 and 44,1 g% of $Na_2 S_2 H_2$. The actual existence is confirmed by statistical studies and by its persistence in pathological sera. Calculating on the basis of these points, the absolute and percentage values of the fractions delimitated by the results are found equivalents to the electrophoretic ones.

The fractioning in 82 pathological sera has been obtained and by means of them it has been confirmed the existence of different types of plasmocitoma, the increase in γ globulin in cirrosis of the liver and in two nephrotic sera the increase in precipitation in the β and α zone. Up to now no pathological curve that does not descend to within the globulin zone, has been observed.

Finally, it may be considered that the functioning with sodium thiosulphate offers theoretical and practical advantages over all salts so far suggested. Theoretically on account of the satisfactory coincidence of the fractions deduced from the inflection points found in its curve, with the electrophoretic ones. Practically because of its great solubility it is possible to prepare its solutions easily and work at room temperature; furthermore it precipitates rapidly and does not disturb the protein determination in the filtrate by Kjeldahl or biuret reaction.

* Departamento de Investigacion del Hospital Municipal de Infecciosos Seccion de Inmunoquimica Barcelona. *Edited by Revista Espanola de fisiologia.*

1952 AWARDS

Hillkowitz Award: Elsbeth Ellis, Medical Research Foundation of Dade County, Inc.

Registry Award: Alma Marie Wittgenstein, Stanford University, Calif.

1st A.S.M.T. Convention Award: Grace Kircher, Wayne County General Hospital, Eloise Michigan.

2nd A.S.M.T. Convention Award: Rachel Lehman, Indiana University School of Medicine, Indianapolis, Indiana.

3rd A.S.M.T. Convention Award: Jean A. Foster, Instructor Medical Laboratory Department, University of the State of New York.

Exhibit Award—(First): Colorado State Society of Medical Technologists.

Exhibit Award—(Second): Arkansas Society of Medical Technologists.

"Award of Merit" from Pathologist Society of Pacific Northwest. Mrs. Jacqueline Bahrenburg, St. Lukes Hospital, Spokane, Washington.

YOUR 1953 ASMT CONVENTION PROGRAM COMMITTEE SPEAKS

Plans are now being made by your Program Committee to arrange a stimulating and interesting program for the annual Convention of ASMT, which will be held in Louisville, Kentucky, June 14-18, 1953. There are many outstanding leaders in every phase of the profession of medical technology among our members, therefore, we urge you to write a paper for the convention program. Please share your knowledge and your experience with your fellow medical technologists. May we hope that a large number of you will submit papers for the various awards which are available:

ASCP Registry Award

ASMT Award

Hillkowitz Memorial Award

Parasitology Award

Some papers not accepted for the program may be suitable for publication in the JOURNAL.

There will be further announcements about the program and awards in later issues of the JOURNAL.

If you have planned to write a paper for the Program, we shall appreciate an early notification of your intention to do so and at least a tentative title. If you have any suggestions for the program, we shall appreciate hearing about it very soon.

Sister Mary Simeonette Savage
Program Committee Chairman

851 South Fourth Street
Louisville 3, Kentucky

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310 Cedar Street, New Haven, Conn.
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3803 Utah Place, St. Louis 16, Mo.
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Medical College of Alabama, Birmingham, Ala.
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678 William Street, Buffalo 6, New York.
Adabelle Noyes, MT(ASCP) 1955
338 Willow Ave., Corte Madera, Calif.
C. Patton Steele, MT(ASCP) 1955
P. O. Box 1020, Bismarck, North Dakota.

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Planck Road, Waterbury, Conn.
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Memorial Hospital, Colorado Springs, Colorado.
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434 Grand Ave., Dayton, Ohio.
Doris Boon, MT(ASCP) 1955
1588 Ansel Road, Cleveland 6, Ohio.

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612 Becker Ave., West Wilmar, Minn.
Howard Moss, MT(ASCP) 1954
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SEMINAR FUND: Four applications for funds have already been approved with several more being considered from the $1,000 grant of the Board of Registry for this year. The programs planned for these Seminars sound very interesting.

A report on the Massachusetts Association Seminar was most enthusiastic. Invitations were issued to technologists in all the New England states. 175 registered with the state presidents of every New England state present except one. One day was devoted to the subject of Clinical Chemistry and one to Hematology with a number of excellent speakers.

The Georgia and South Carolina Societies held a joint Seminar on April 5th in Savannah, Georgia; Illinois Society presents a Seminar on March 30th in Champaign and Washington State has planned a Chemistry Seminar for April 26th with technologists from Idaho and Oregon invited.

Your state society alone or jointly with surrounding state societies can arrange such an educational meeting, too. Plan now for a fall Seminar.

How to obtain grants—Write for an application form from the Chairman of the Seminar Fund:

Mary Frances James,
c/o Medical College of Alabama,
Birmingham, Alabama.

Allow a few weeks time for the handling of these requests by the committee. Be prepared to outline the program with anticipated financial needs itemized.

Rules governing its use—The Seminar must be planned as an educational meeting apart from the annual state meeting.

The funds can be used to pay the speakers and/or their traveling expenses, rental on the meeting place, films and projectors.

The fund cannot be used for defraying the expense of advertising, printing of programs or entertainment.